

**REMARKS**

Claims 25-36 are all the claims pending in the application.

Claims 25-36 have been amended to remove specific reference to the apparatus claimed therein as either a “measuring” apparatus as in claim 25, or as a “quantitative” apparatus as in claims 26-35. Claim 25 specifically states that the apparatus is “for measuring a value.” Thus, the inclusion of the term “measuring” or quantitative” in the preamble is only redundant.

Claim 25 has also been amended to more clearly refer to the measuring being “corrected for” rather than “based on” a value detected from the first label. Support for this change can be found in the last line of the claim where it states that the “quantity of said organism-originated substance bound to said specific binding substance, based on the detected level of said second labeling signal, corrected for the detected level of said first labeling signal.”

Claim 26, 27 and 28 have been amended to correct obvious grammatical errors.

Claims 27 and 28 have also been amended to more clearly state that the characteristic values are “corrected values” as first recited in independent claim 25.

Thus, no new matter has been added and entry of the Amendment is earnestly solicited.

**I. Objection of claims under 37 C.F.R. §1.75(c)**

At page 2 of the Office Action, paragraph 2, claims 28, 31, 32, 35 and 36 are objected to as improper dependent claims under 37 C.F.R. §1.75(c).

The Examiner alleges that the cited claims fail to further limit the subject matter of the claims from which they depend. Specifically, the Examiner contends that claim 28 does not further limit claim 26, claims 31 and 35 do not further limit claim 27, and claims 32 and 36 do not further limit claim 28.

A. Claim 28

In response, Applicant asserts that claim 28 further limits the inventive apparatus recited in claim 26 by defining the means of performing the quantitative analysis. Therefore, in contrast to the Examiner's rejection, claim 28 does in fact set forth a further limitation to claim 26.

However, in order to further the prosecution of the application, Applicant has amended claim 28 to more clearly recite the additional limitation recited in claim 28. Applicant has amended the claim to describe the correction value as a value "calculated from information about 1) the length of the cDNA polynucleotide and 2) the relative frequency of the first labeling substance within each cDNA polynucleotide used in the method."

Accordingly, Applicant respectfully requests reconsideration and withdrawal of this portion of the objection.

B. Claims 31, 32, 35 and 36

Contrary to the Examiner's position, Applicant asserts that each of claims 31 and 35 clearly further limit claim 27. Claim 27 depends from claim 25, and claim 25 merely recites a "first labeling substance." Claim 31 further defines the first labeling substance as a fluorescent dye and claim 35 further defines it as a radioactive isotope.

Similarly, Applicant asserts that each of claims 32 and 36 clearly further limit claim 28. Claim 28 depends from claim 26, which in turn depends from claim 25, and claim 25 merely recites a "first labeling substance." Claim 32 further defines the first labeling substance as a fluorescent dye and claim 36 further defines it as a radioactive isotope.

Thus, in contrast to the Examiner's position, claims 31 and 35 further limit claim 27, and claims 32 and 36 further limit claim 28. Accordingly, Applicant respectfully requests reconsideration and withdrawal of this portion of the objection.

**II. Rejection of claims under 35 U.S.C. §112**

A. Claim 25

At page 3 of the Office Action, paragraph 4, claim 25-36 are rejected as being indefinite.

The Examiner is unclear as to meaning in claim 25 of the phrase "for measuring a value detected from a second label based on a value detected from a first label."

In response, Applicant has amended claim 25 to more clearly recite the invention.

Amended claim 25 states that the value detected from the second label is "corrected for" based on the value detected from the first label. Thus, it is now clear that the value detected from a first label serves to take into account and correct for, or control for, variations in the quantity of specific binding substances (detected by the first label) disposed on the carrier.

Therefore, Applicant respectfully requests reconsideration and withdrawal of this portion of the rejection.

B. Claim 27

At page 3 of the Office Action, paragraph 5, claim 27 is rejected as being indefinite.

The Examiner claims that there is insufficient antecedent basis for "cDNA polynucleotides" in the claim.

In response, Applicant has amended claim 27 to recite "specific binding substance" in place of "cDNA polynucleotides."

Therefore, Applicant respectfully requests reconsideration and withdrawal of this portion of the rejection.

**III. Rejection of claims under 35 U.S.C. § 102**

At page 3 of the Office Action, paragraph 7, claims 25 and 29 are rejected under 35 U.S.C. §102(b) as being anticipated by Larin et al. (*Nuc. Acid Res.*, 1994).

The Examiner asserts that Larin et al. teaches a method of analyzing multiple probes by FISH to metaphase chromosomes using two different labeled signals and an imager system as an analyzing means. The Examiner contends that the propidium iodide (PI) counterstain qualifies as the first labeled signal, the fluorescent biotin-avidin complex as the second labeled signal, and the BioRAD optical system as the analyzing means. Thus, the Examiner asserts that Larin et al. teaches all of the limitations of claims 25 and 29.

In response, Applicant asserts that Larin et al. does not teach each of the limitations of claims 25 and 29. Specifically:

- Larin et al. does not provide a “means for detecting a level of a first labeling signal,”
- the PI does not label “known different specific binding substances,”
- the metaphase chromosomes do not qualify as “substances disposed at predetermined positions on the carrier,” and
- the analysis performed in Larin et al. is not “corrected for a value detected from a first label.”

Each of these points is discussed, in turn.

A. The Examiner considers the PI counterstain a first labeled signal. Accepting that contention, Applicant notes that PI is an intercalating agent, inserting between the bases of DNA,

and fluorescing under ultraviolet light. In FISH, the PI is needed to observe and identify the specific chromosomes under analysis.

However, even if the PI is considered a first labeled signal, the system described in Larin et al. does not provide a means for detecting a level of PI. There is no evidence in Larin et al. that the BioRAD optical system measures any quantitative difference in the level of fluorescence emitted by the PI. The general staining function of PI is simply to assist in the karyotype determination of the metaphase chromosome spread.

B. The present invention recites the first labeling substance as labeling “known specific binding substances” (claim 25). Applicant discloses at page 8, lines 10-12, of the specification that “known” means that the base sequence and length of the nucleic acid sequence is known. In contrast, there is no indication in Larin et al. that the sequence and length of the chromosomes are known. Thus, at the time of Applicant’s invention, whole chromosomes did not qualify as “known specific binding substance” as defined by the Applicant.

C. Applicant defines at page 8, line 13-16, of the specification that “specific binding substances disposed at predetermined positions on the carrier” means that one kind of specific binding substance has been disposed for each position. Applicant asserts that Larin et al. does not teach this element. Larin et al. merely teaches a metaphase chromosome suspension spread onto a glass slide or glass plate so as to completely cover the slide/plate (p. 3690). Larin et al. does suggest that the chromosomes may be applied to predefined areas of the slide, and come from different sources (Fig. 1).

Further, Applicant asserts that chromosome suspensions prepared as described in Larin et al. contain a mixture of specific binding substances (the chromosomes). In other words, a chromosome solution dropped on to a predefined area does not contain one kind of specific binding substance, it contains more than one different chromosome. Thus, although the chromosome mixture may be applied to predetermined areas of a slide, the position of each particular chromosome within that area is not predefined but rather is randomly generated.

D. Larin et al. teaches a method of FISH analysis which consists entirely of optically observing and manually counting the signals generated by the hybridization of centromere and YAC probes to metaphase chromosomes. The PI counterstain is used in Larin et al. to produce a contrasting red color and functions to discriminate particular metaphase chromosome spreads which the observer then determines are optimal for analysis. Larin et al. does not teach the Applicant's claim limitation that the level of the second labeling signal is "corrected for" the value of the first labeling signal. Larin et al. does not determine a quantitative measurement of the PI stain. The chromosomes are simply stained with PI in order to coordinate the identification of appropriate metaphase spreads and to verify the identity of particular chromosomes in which the specific probes are bound.

Base claim 25 explicitly recites that the quantitative analysis performed in Applicant's invention corrects for the detected value of the first labeling signal. The signal generated by the first labeling substance is quantified and becomes an integral part of the calculation of the level of organism-originated substance. Larin et al. simply does not teach measuring the value of PI fluorescence nor correction of the quantity of specific probes.

Thus again, Applicant asserts that Larin et al. does not teach each of the limitations of claims 25 and 29, and respectfully requests reconsideration and withdrawal of this rejection.

**IV. Rejection of claims under 35 U.S.C. § 103**

A. Claims 25 and 33

At page 4 of the Office Action, paragraph 9, claims 25 and 33 are rejected under 35 U.S.C. §103(a) as obvious over Arnold et al. (Mol. Endocrinol., 1995) in view of Brown et al. (U.S. Pat. No. 5,849,920; the “’920 patent”).

The Examiner states that Arnold et al. teaches protein analysis by radioactive labeling, chemiluminescence, and x-ray film detection, and that the ’920 patent reports the use of a densitometer for scanning x-ray films. The Examiner contends that it would have been *prima facie* obvious to one having ordinary skill in the art to modify and combine the teachings of the two methods and construct Applicant’s claimed apparatus.

The Examiner contends that the  $^{32}\text{P}$ -labeled hER qualifies as the “first labeling signal with known different specific binding substances,” while the ECL visualization of hER qualifies as the “second labeled signal.” The Examiner acknowledges that the Arnold et al. reference does not disclose an apparatus to scan an x-ray film.

In response, Applicant asserts that claims 25 and 33 are not obvious over Arnold et al., in view of the ’920 patent, for the following reasons.

First, Applicant asserts that the combination of detection by chemiluminescence along with the analysis of x-ray film by densitometry does not lend itself to the type of precise quantification disclosed in the Applicant’s invention. Although ECL chemiluminescence used in

conjunction with western blot is a common research technique, its popularity stems from factors related to simplicity, cost and convenience. ECL is a popular alternative to methods which rely on radioisotopes. Despite its growing popularity, Applicant contends that there are inherent problems with ECL which limit its use. Specifically, the ECL technique does not lend itself to accurate quantification of the level of a signal.

Second, the limitations of ECL are further compounded by its combination with x-ray film detection. It is well known and documented in the art that x-ray film contains a very narrow linear range. X-ray film inherently possesses a very limited range in which the amount of exposure (in other words the degree of blackness) correlates accurately with the amount of signal producing the exposure. The film is limited on the low end because very weak signals have to overcome an initial "step" in order to cause the film's emulsion to react. The film is limited on the high end because very strong signals saturate the film's emulsion. It is also well known in the art that multiple exposures of an x-ray film, along with several signal concentrations, are often necessary to permit data integration within the linear range of film exposure (*see*, the '920 patent '920, col. 23, line 43-48).

Applicant therefore asserts, that in contrast to the Examiner's position that it would have been obvious to combine Arnold et al. with the '920 patent to arrive at the present invention, such a combination will not produce the precise analysis and quantification of labeled signal as disclosed by the present invention.

Applicants also contend that the method of Arnold et al. describes procedures that are heavily dependant on manual intervention. Thus, the combination of the two references as the

Examiner suggests does not lend itself to the type of automatic operation disclosed by the Applicant's invention. Arnold et al. describes SDS-gel electrophoresis, western blot, autoradiographic detection of a signal generated from a <sup>32</sup>P-labeled phosphoprotein, followed by detection of signal generated using antibodies and ECL, all involving significant manual operation. Arnold et al. discloses further manual procedure by stripping the first probe from the western blot membrane and reprobing with a second probe.

Another significant problem of adapting Arnold et al. with the '920 patent is that if the initial <sup>32</sup>P-generated signal is significantly strong, it can interfere with the second ECL generated signal. Arnold et al. does not provide a means to discriminate between the two signals. Both the first labeled signal and the second labeled signal are detected by a single source, that is x-ray film.

A further problem is that Arnold et al. is limited to the detection of a protein (hER) in which there are known antibodies. And, it is further limited to the detection of proteins which can be *in vitro* phosphorylated.

Thus again, Applicant asserts that the teachings of Arnold et al., combined with the teachings of the '920 patent, would not make obvious, or even suggest, the present invention. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

B. Claims 25-32.

At page 6 of the Office Action, paragraph 10, claims 25-32 are rejected under 35 U.S.C. §103(a) as being unpatentable over Brown et al., (U.S. Pat. No. 5,807,522; "the '522 patent") in view of Cardullo et al. (PNAS, 1988).

The Examiner asserts that the '522 patent teaches cDNA microarrays hybridized with dual-labeled nucleic acid probes and that Cardullo et al. teaches a method and apparatus for the detection of nucleic acid hybridization. The Examiner contends it would have been obvious to combine the teachings of the two in order to produce the present claimed invention.

The Examiner acknowledges that neither the '522 patent nor Cardullo et al. teaches fluorescence labeled cDNA immobilized on a solid support (corresponding to Applicant's claim limitation of a first labeled binding substance disposed on a carrier). However, the Examiner alleges that it would have been obvious to construct a system whereby fluorescence-labeled cDNA is substituted for unlabeled cDNA in the '522 patent microarray, and then combined with the FRET technique of Cardullo et al. to detect and measure a hybridization signal (from a second labeled substance).

In response, Applicant asserts that the subject matter recited in claims 25-32 would not have been obvious over the teachings of the '522 patent in view of Cardullo et al. for the following reasons.

Applicant first asserts that it is not obvious to replace the detection method of the '522 patent with the method of Cardullo et al. Indeed, neither of the two references provide any suggestion to use FRET with nucleic acid fixed to a solid support. The FRET technique disclosed in Cardullo et al. is performed entirely in solution hybridization studies. At page 8790, Cardullo et al. state: "We report here that FRET provides a useful means for detection of nucleic acid hybridization in solution." In FRET, the hybridization between complementary strands of DNA takes place in solution in a cuvette. Hybridization is measured with a spectrophotometer

by following changes in fluorescence resulting from proximity-induced dipole interactions between two fluorophores. There is no suggestion in Cardullo et al. that FRET could be adapted to measure hybridization of nucleic acid on a solid support.

Second, Cardullo et al. teaches away from the application of FRET to measure nucleic acid hybridization as disclosed by the present invention. The signals generated during hybridization by the dual fluorescence labels are highly temperature sensitive. At page 8792 and in Fig. 4 of Cardullo et al., the authors disclose the effects of temperature on fluorescence intensity. The data indicated that the measured values for rhodamine enhancement and fluorescein quenching were highly variable over a relatively narrow temperature range. One skilled in the art would recognize the difficulty in adapting FRET to the analysis of nucleic acid hybridization while still obtaining the type of accurate and quantitative measurement that the present invention demands.

Furthermore, at page 8792, Cardullo et al. states: "On the basis of these studies and our experience so far, it is not apparent that FRET offers increased sensitivity over existing methods for detecting nucleic acid hybridization." The authors then go on to suggest that FRET could be useful for detecting qualitative hybridization *in vivo*, such as the observation of viral infection and replication in living cells. One skilled in the art would therefore find no motivation to adapt FRET to the quantitative analysis of hybridization as claimed by the present invention.

Thus, Applicant asserts that there is no motivation to substitute fluorescence-labeled cDNA for unlabeled cDNA in the '522 patent microarray. One skilled in the art would not

expect that fluorescence-labeled cDNA fixed to a carrier would perform as the Examiner contends in a FRET type of analysis.

Therefore again, Applicant asserts that the subject matter recited in claims 25-32 would not have been obvious over the teachings or suggestions of the '522 patent in view of Cardullo et al. Accordingly, Applicant respectfully requests reconsideration and withdrawal of this rejection.

**V. Conclusion**

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

Applicant hereby petitions for any extension of time which may be required to maintain the pendency of this case, and any required fee, except for the Issue Fee, for such extension is to be charged to Deposit Account No. 19-4880.

Respectfully submitted,



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**APPENDIX**  
**VERSION WITH MARKINGS TO SHOW CHANGES MADE**  
**IN THE CLAIMS:**

**The claims are amended as follows:**

25. (Twice amended) ~~An~~ ~~A measuring~~ apparatus for measuring a value detected from a second label ~~corrected for based on~~ a value detected from a first label comprising:

a first detection means for detecting a level of a first labeling signal emitted by a first labeling substance, which labels a plurality of known different specific binding substances respectively disposed at a plurality of predetermined positions on a carrier of a test piece, for each of said plurality of predetermined positions;

a second detection means for detecting a level of a second labeling signal emitted by a second labeling substance, which differs from said first labeling substance and labels an organism-originated substance bound to said specific binding substance, for each of said plurality of predetermined positions; and

an analyzing means for measuring a quantity of said organism-originated substance bound to said specific binding substance, based on the detected level of said second labeling signal, corrected for the detected level of said first labeling signal.

26. (Twice amended) The ~~quantitative~~-apparatus as set forth in claim 25, wherein said specific binding ~~substance is a cDNA polynucleotides~~ ~~substances are cDNA polynucleotides~~.

27. (Twice amended) The ~~quantitative~~-apparatus as set forth in claim 25, wherein said analyzing means ~~performing further performs~~ said measurement, ~~utilizes a correction value~~

calculated for each specific binding substance used in the method~~based on a characteristic value related to cDNA polynucleotides.~~

28. (Twice amended) The ~~quantitative~~ apparatus as set forth in claim 26, wherein said analyzing means performing further performs said measurement, <sup>✓</sup>utilizes a correction value calculated from information about 1) the length of the cDNA polynucleotide and 2) the relative frequency of the first labeling substance within each cDNA polynucleotide used in the method~~based on a characteristic value related to cDNA polynucleotides.~~

29. (amended) The ~~quantitative~~ apparatus as set forth in claim 25, wherein said first labeling substance for said specific binding substances is a fluorescent dye.

30. (amended) The ~~quantitative~~ apparatus as set forth in claim 26, wherein said first labeling substance for said specific binding substances is a fluorescent dye.

31. (amended) The ~~quantitative~~ apparatus as set forth in claim 27, wherein said first labeling substance for said specific binding substances is a fluorescent dye.

32. (amended) The ~~quantitative~~ apparatus as set forth in claim 28, wherein said first labeling substance for said specific binding substances is a fluorescent dye.

33. (amended) The ~~quantitative~~ apparatus as set forth in claim 25, wherein said first labeling substance for said specific binding substances is a radioactive isotope.

34. (amended) The ~~quantitative~~ apparatus as set forth in claim 26, wherein said first labeling substance for said specific binding substances is a radioactive isotope.

35. (amended) The ~~quantitative~~ apparatus as set forth in claim 27, wherein said first labeling substance for said specific binding substances is a radioactive isotope.

36. (amended) The quantitative apparatus as set forth in claim 28, wherein said first labeling substance for said specific binding substances is a radioactive isotope.